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The influence of three barrier membranes on modeling and incorporation of autologous onlay bone grafts in rats. An evaluation by transversal microradiography

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ABSTRACT

Objectives: To determine whether covering an autologous bone grafts with three different barrier membranes prevents graft resorption, and to compare these membranes to each other.

Design: In 192 rats a standardised 4.0 mm diameter bone graft was harvested from the right mandibular angle and transplanted to the left. Membranes used to cover the grafts were a new poly(DL-lactide-ε-caprolactone) membrane, a collagen and expanded polytetrafluoroethylene membrane. The controls were left uncovered. Graft resorption and incorporation were measured with transversal microradiography (TMR) in the four groups at 2, 4 and 12 weeks. Data were analysed using multiple regression analyses.

Results: Overall, there were no differences in modeling with resorption between the four groups. ePTFE at 12 weeks showed a lower mineralization ratio and graft height of the graft as compared to the other groups. The mean graft incorporation was progressive and nearly identical from 2 to 12 weeks in all groups.

Conclusions: Membranes have an equal effect on bone graft modeling and resorption as found in non-covered controls. Therefore, the indication to use a barrier membrane to prevent bone modeling with resorption and enhance incorporation of autologous onlay bone grafts is disputable.

1. Introduction

Guided bone regeneration is a commonly known technique for alveolar ridge augmentation in maxillofacial surgery. The technique has been proven to promote bone regeneration in bony defects when covered by a barrier membrane.[1,2] When an autologous bone graft is used to augment the alveolar ridge, it can be covered with similar barrier membranes. The bone graft serves as a scaffold and carrier for living cells. The barrier membrane on top of the graft is expected to prevent bone modeling with subsequent resorption of the bone graft and the membrane may improve the predictability of the augmentation by enhancing bone graft incorporation.[3] However, due to weak evidence,[4] it is still unclear if a barrier membrane should be used to cover the augmented site.[5,6]

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Although different barrier membranes have been developed over the years, the ideal barrier membrane is not yet available. Some reasons are poor space maintaining capacities[7] and the necessity of secondary removal. An optimal membrane should be biocompatible, occlusive, synthetic, space maintaining, clinically manageable, and degradable.[8–10]

A new poly(DL-lactide-ε-caprolactone) (PDLLCL) barrier membrane[11] might have advantages when compared to the currently applied barrier membranes. This membrane has been shown to be biocompatible and non-cytotoxic.[11] The polymer is already applied in a commercially available nerve guide (Neurolac®, Polyganics, Groningen, The Netherlands).[12] Based on its chemical composition and size it can be expected to be occlusive, space maintaining and flexible enough to adapt to the contour of the cortical bone and graft.

In guided bone regeneration studies, radiology,[13,14] histology[15,16] and histomorphometry[17] are common methods to evaluate bone volume and to specify the various cell types involved. Both microradiography and micro-CT proved to be accurate methods in graft studies compared to histology.[18] However, bone mineralization and resulting density cannot be measured validly with these methods. Transversal microradiography (TMR) is an accurate method of measuring mineral content in a thin irradiated cross section of a sample.[19] This method has proven to be valid, precise, and useful for measuring mineral loss.[20–22]

The objective of this study was first to study the preventive effect of a PDLLCL, collagen and expanded polytetrafluoroethylene (ePTFE) membrane on resorption of autologous onlay bone grafts in the rat mandible, and second the effect of the membranes on graft incorporation.

2. Material and methods

2.1. Surgical procedure

In the right mandibular angle of 192 male Sprague–Dawley rats (mean weight 364 ± 17 g SD, range 320–407 g) a standardised 5.0 mm circular defect was drilled with a trephine[13,23] and the obtained bone graft (4.0 mm diameter) was transplanted to the buccal side of the contralateral mandibular angle and fixed with a slowly degradable suture (Monocryl®, Ethicon, Johnson & Johnson, Amersfoort, The Netherlands) through a central 1 mm hole in the graft.

The rats were assigned to one of four groups: three membrane groups and one control group, in which no membrane was used. The membranes used were (1) a copolymer sheet composed of 67–69% DL (15–85)-lactide and 31–33% ε-caprolactone (poly(DL-lactide-ε-caprolactone) (Vivosorb®, Polyganics, Groningen, The Netherlands), (2) a porcine collagen membrane (Bio-Gide®, Geistlich, Wolhusen, Switzerland), and (3) an expanded polytetrafluoroethylene membrane (ePTFE, Gore-Tex®, W.L. Gore & Associates, Flagstaff, USA).

One side of the PDLLCL-membranes was rough. These membranes were applied with this side faced to the bone to optimize integration and positioning.

The wound was closed in layers using resorbable sutures (Vicryl Rapide 4-0, Ethicon, Johnson & Johnson, Amersfoort, The Netherlands). Postoperative pain relief (a single dose of Caprofen (4.0 mg/kg) and Temgesic (0.03 mg/kg) was administered and the diet was composed of standard laboratory food.

After 2, 4 and 12 weeks, rats were anaesthetised by nitrous-oxygen-isoflurane inhalation anaesthesia and sacrificed by an intracardially injected overdose of pentobarbital, after which the mandibles were explanted and fixed in 4% phosphate buffered formaline solution.

The study protocol was approved by the Animal Studies Review Committee, and in accordance with Institutional Guidelines (University Medical Center Groningen, The Netherlands).

2.2. Preparation of samples and transversal microradiography (TMR)

The specimens were placed in a metal mould and embedded in polymethylmethacrylate (PMMA). Thereby, blocks with standardised dimensions were obtained to facilitate precise cutting and to prevent the samples from drying. X-rays were taken to determine the exact location of the grafts. Through the center of the graft, three cuts were made in the transversal plane by a circular saw blade (Buehler Diamond Wafering Blade (11-4244), diameter 10.2 cm × 0.3 mm, USA) to create two cross-sections with a standardised thickness of 0.50 mm (Fig. 1).

The sections were placed between a 35 mm film (Fuji B and W POS/71337) and an X-ray source (Philips PW 1730, Eindhoven, The Netherlands) and exposed for 18 s with a tube charge of 25 kV and 25 mA to obtain the transveral microradiographs.[22] After film development, a stereo microscope (Wild/Leitz M7 S, Heerbrugg, Switzerland; magnification 10×) and a CCD camera (Scion Corporation CFW 1312 M, Frederick, MD, USA) were used to digitize the images. By means of a frame grabber the images were stored on a PC (resolution: 256 grey values/1360 × 1024 pixels).

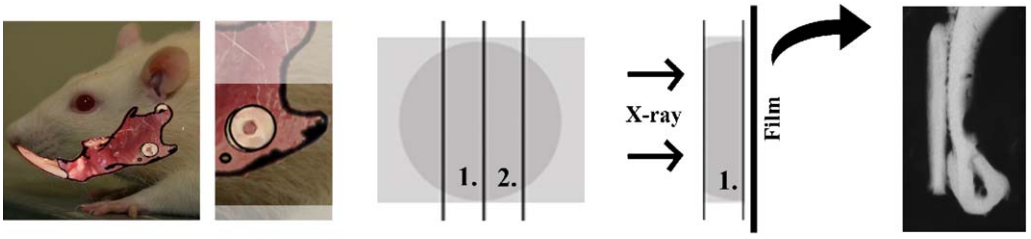

Fig. 1 – Preparation of samples and TMR. Post mortem three cuts were made in the transversal plane through the center of the graft, located at the left mandibular angle, to create two cross-sections (1 and 2). With an X-ray source transversal microradiographs were taken on film. After film development the images were magnified and digitized.

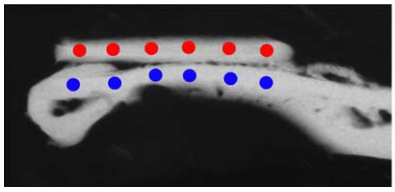

Fig. 2 – Graft modeling with resorption was measured as mineralization ratio, i.e., the ratio of the mean grey value of the bone graft in comparison to the mean grey value of the original underlying mandibular bone. The mean grey value in the two areas was obtained by selecting twelve spots on each radiograph; six within the bone graft and six within the original bone.

2.3. *Measurement of graft modeling with resorption and graft incorporation*

All measurements were performed twice under blind conditions and were averaged. Graft resorption was measured as mineralization ratio as well as graft height. The mineralization ratio was determined by dividing the mean grey value of the bone graft by the mean grey value of the original underlying mandibular bone. The mean grey value of the two areas was obtained by selecting twelve spots on each radiograph; six within the bone graft and six within the original bone (Fig. 2). The measurements were performed using image analysis software (Optical Bone Calculations, J. de Vries, University Medical Center Groningen, The Netherlands). Graft height was measured using image analysis software (Scion Corporation CFW 1312 M, Frederick, MD, USA). A line was drawn between the center at the buccal side of the graft and the center of the lingual side of the graft; the length in pixels was measured automatically.

Furthermore, graft incorporation, which was defined as a bony connection between graft and mandible, was measured.[13] The percentage of incorporation was defined as the length of the incorporated part of the graft divided by the total length of the graft. When 0–25% of the graft was incorporated a score of 1 was assigned, and a score 2, 3 and 4 were assigned in case of 26–50%, 51–75% or 76–100% of incorporation, respectively.

2.4. *Statistical analyses*

The sample size was determined by a power analysis based on a 90% power with a 0.05 two sided significance level, a 40% difference in graft size between a membrane treated group and a non treated control, and a mean standard deviation of 29%.[3,5] For each graft a mean score per variable was calculated by averaging the outcomes of the two corresponding sections. In a multiple regression analysis model the effect of the independent variables 'group' (i.e., control, PDLLCL, collagen, ePTFE) and 'time' (i.e., 2, 4 and 12 weeks) and interactions between these variables on graft modeling with resorption and graft incorporation was studied.

3. Results

During surgery six rats died. In another six rats the graft fractured during drilling. These samples were excluded from the study. Due to problems during sectioning an additional number of samples had to be excluded. It resulted in a median group size of 14 samples (range 11–15) for mineralization, height and incorporation measurements.

The mean graft modeling with resorption as mineralization ratio, i.e., the ratio of the mean grey value of the bone graft in comparison to the mean grey value of the original underlying mandibular bone, is presented in Table 1. The mean graft modeling with resorption as graft height is presented in Table 2. Table 3 presents graft incorporation. In Tables 1 and 2 is observed that ePTFE at 12 weeks shows a lower mineralization ratio and less graft height compared to the other membranes and control. Table 3 shows more incorporation in PDLLCL at 2 weeks compared to the other groups.

The regression analyses of the graft modeling with resorption measured as mineralization ratio and as graft height as well as graft incorporation are summarized in Table 4. Model 1 is a regression model without the correction for possible effect modification (interaction effects). Model 2 is a regression model with correction for effect modification of time and membrane (i.e., PDLLCL, collagen or ePTFE), respectively. Both models are presented to give the reader information about the relative effect of the coefficients with and without correction for effect modifications, as interaction

Table 1 – Graft modeling with resorption as mineralization ratio, i.e., the ratio of the mean grey value of the bone graft in comparison to the mean grey value of the original underlying mandibular bone (Fig. 2).

	2 wks (95% CI) (g/m)	4 wks (95% CI) (g/m)	12 wks (95% CI) (g/m)
Control	0.94 (0.88–1.00) (N = 11)	0.97 (0.93–1.01) (N = 14)	0.95 (0.93–0.97) (N = 15)
PDLLCL	0.94 (0.91–0.97) (N = 14)	0.95 (0.91–0.99) (N = 14)	0.90 (0.87–0.93) (N = 14)
Collagen	0.92 (0.88–0.96) (N = 14)	0.96 (0.94–0.98) (N = 13)	0.92 (0.87–0.97) (N = 12)
ePTFE	0.94 (0.92–0.96) (N = 14)	0.99 (0.97–1.01) (N = 14)	0.82 (0.77–0.87) (N = 14)

CI: confidence interval; N: number of evaluated samples; PDLLCL: poly(DL-lactide-ε-caprolactone); ePTFE: expanded polytetrafluoroethylene; g: graft bone; m: mandibular bone.

Table 2 – Graft modeling with resorption as graft height measured in the center of the grafts scored in mm. A line was drawn between the center at the buccal side of the graft and the center of the lingual side of the graft; the length in pixels was measured automatically.

	2 wks (95% CI) (mm)	4 wks (95% CI) (mm)	12 wks (95% CI) (mm)
Control	0.54 (0.43–0.65) (N = 11)	0.56 (0.36–0.76) (N = 14)	0.44 (0.32–0.56) (N = 15)
PDLLCL	0.44 (0.35–0.53) (N = 14)	0.40 (0.36–0.44) (N = 14)	0.41 (0.33–0.49) (N = 14)
Collagen	0.28 (0.24–0.32) (N = 12)	0.38 (0.32–0.44) (N = 13)	0.40 (0.31–0.49) (N = 14)
ePTFE	0.28 (0.24–0.32) (N = 14)	0.50 (0.39–0.61) (N = 14)	0.19 (0.15–0.23) (N = 14)

CI: confidence interval; N: number of evaluated samples; PDLLCL: poly(DL-lactide-ε-caprolactone); ePTFE: expanded polytetrafluoroethylene.

Table 3 – Mean graft incorporation. When 0–25% of the graft was incorporated a score of 1 was assigned, and a score 2, 3 and 4 were assigned in case of 26–50%, 51–75% or 76–100% of incorporation, respectively.

	2 wks (95% CI) (1–4)	4 wks (95% CI) (1–4)	12 wks (95% CI) (1–4)
Control	1.18 (0.83–1.53) (N = 11)	2.18 (1.55–2.81) (N = 14)	3.27 (2.70–3.84) (N = 15)
PDLLCL	2.36 (1.97–2.75) (N = 14)	2.86 (2.41–3.31) (N = 14)	3.36 (2.95–3.77) (N = 14)
Collagen	1.17 (1.03–1.31) (N = 12)	2.42 (1.98–2.86) (N = 13)	2.96 (2.37–3.55) (N = 14)
ePTFE	1.79 (1.33–2.25) (N = 14)	2.29 (1.87–2.71) (N = 14)	3.29 (2.99–3.59) (N = 14)

CI: confidence interval; N: number of evaluated samples; PDLLCL: poly(DL-lactide-ε-caprolactone); ePTFE: expanded polytetrafluoroethylene.

may dramatically change the value of the crude coefficients. The regression analyses showed that graft resorption as mineralization ratio was lower in the ePTFE groups compared to the other membrane groups and control. The graft height as depicted in model 2 increased only in the collagen group, whereas model 1 shows a decreasing graft height in this group. No differences were seen between the other groups. Based upon model 2, graft incorporation in the other groups increased more compared to PDLLCL, whereas model 1 showed that PDLLCL increased more compared to other membranes. Overall, equal results were obtained in membranes and control groups, although minor differences were observed.

4. Discussion

The results of the present study indicate that the barrier membranes studied do not have a preventive effect on onlay bone graft resorption in the rat mandible. Furthermore, the results do not support the statement that membranes would have a positive effect on graft incorporation. Conclusions in other studies were conflicting.[5,24–26] Based on the results of a systematic review of the literature, it was concluded that the best available evidence does not support membrane use to prevent graft resorption.[4]

In the present study graft modeling with resorption was evaluated as mineralization ratio and graft height. The mineralization was measured as a ratio between the mean grey values of the bone graft and of the original underlying mandibular bone. An absolute value of mineralization would have been more appropriate. However, calibration and validation of mineral content of different types of bone related to grey values of microradiographs is difficult. Therefore, in the present study the grey value of the original underlying original bone was chosen as 100% mineralization. Theoretically the original underlying original bone is more or less constant. However, especially in the 12 weeks' samples mineral was lost in the original underlying bone that possibly would explain the higher than expected mineralization ratios. The loss of mineral and volume of original underlying bone was also seen in 3D analyses of the same samples[27] and found in other research.[28] A higher osteoclast-activity due to a better perfusion in host bone compared to grafts, consisting of predominantly cortical bone might cause the resorption. Revascularization, incorporation and modeling of these grafts might rely on previous host bone resorption.[28]

It was expected that graft resorption with mineral loss, demonstrated by a decreasing ratio, would be observed from 2 to 12 weeks. However, this was only seen in the ePTFE group (Tables 1 and 4). Care was taken that the mineralization of the underlying original bone was measured in areas unaffected by modeling with resorption. The mineralization ratio and graft height of ePTFE at 12 weeks was lower compared to other groups (Tables 1 and 2). It is known that ePTFE exposure to the oral environment during healing has a major negative effect on guided bone regeneration around dental implants because of infection.[29] However, in the present study no exposure of the ePTFE membranes was observed.

Graft height increased only in the collagen groups from 2 to 12 weeks (Table 4). However, model 1 shows a decreasing graft height in the collagen group and the amount of graft bone at each occasion is smaller than or similar to the other groups (Table 2). Therefore the clinical relevance of the effect modification between time and collagen is small. A notable finding was the rather large graft height in the control groups compared to the membrane groups (Table 2). Unrestrained growth of bone in the graft surrounding region was seen in some control samples, which might explain the high means and large confidence intervals in the controls. The smaller confidence intervals seen overall in the membrane-treated groups suggest a more predictable treatment outcome by membrane application. This is in line with results in other studies.[3,6] The variations in graft height might be a result of

Table 4 – Linear regression models of graft modeling with resorption as mineralization ratio, graft modeling with resorption as graft height and graft incorporation, respectively. Model 1 is a regression model without the correction for interaction effects, model 2 with correction for interaction effects.

Model	Coefficients					
	Mineralization ratio		Graft height		Graft incorporation	
	B (95% CI)	Significance	B (95% CI)	Significance	B (95% CI)	Significance
1						
Constant	1.001 (0.962 to 1.040)	0.000	0.537 (0.437 to 0.638)	0.000	0.656 (0.215 to 1.097)	0.004
Control (time)	−0.022 (−0.036 to −0.007)	0.004	−0.012 (−0.050 to 0.025)	0.515	0.789 (0.623 to 0.954)	0.000
PDLLCL	−0.027 (−0.060 to 0.007)	0.115	−0.096 (−0.182 to −0.009)	0.030	0.624 (0.244 to 1.003)	0.001
Collagen	−0.025 (−0.059 to 0.009)	0.155	−0.155 (−0.243 to −0.067)	0.001	−0.043 (−0.430 to 0.343)	0.825
ePTFE	−0.044 (−0.078 to −0.011)	0.010	−0.189 (−0.276 to −0.103)	0.000	0.219 (−0.161 to 0.599)	0.257
2						
Constant	0.951 (0.885 to 1.017)	0.000	0.625 (0.453 to 0.797)	0.000	0.118 (−0.638 to 0.874)	0.758
Control (time)	0.002 (−0.027 to 0.032)	0.876	−0.054 (−0.131 to 0.023)	0.165	1.045 (0.708 to 1.381)	0.000
PDLLCL	0.024 (−0.065 to 0.114)	0.590	−0.182 (−0.416 to 0.052)	0.126	1.739 (0.712 to 2.766)	0.001
Collagen	−0.012 (−0.104 to 0.080)	0.794	−0.396 (−0.637 to −0.156)	0.001	0.288 (−0.769 to 1.345)	0.591
ePTFE	0.082 (−0.007 to 0.171)	0.071	−0.215 (−0.449 to 0.019)	0.072	0.834 (−0.193 to 1.861)	0.111
Interaction: Time × PDLLCL	−0.024 (−0.065 to 0.016)	0.234	0.041 (−0.065 to 0.147)	0.444	−0.545 (−1.010 to −0.079)	0.022
Interaction: Time × collagen	−0.006 (−0.047 to 0.036)	0.790	0.117 (0.009 to 0.225)	0.034	−0.155 (−0.630 to 0.319)	0.519
Interaction: Time × ePTFE	−0.062 (−0.102 to −0.022)	0.003	0.011 (−0.095 to 0.117)	0.844	−0.295 (−0.760 to 0.171)	0.213

CI: confidence interval; PDLLCL: poly(DL-lactide-ε-caprolactone); ePTFE: expanded polytetrafluoroethylene.

differing initial heights. Therefore it would have been preferable to measure graft height during surgery.

The mean incorporation was progressive from 2 to 12 weeks in all groups. Most incorporation of the graft was seen in the PDLLCL groups compared to the other groups. However, since model 2 (Table 4) showed that there was effect modification between PDLLCL and time, incorporation of the graft beneath the PDLLCL membrane was significantly altered within the time-frame of this study, suggesting a decreasing incorporation. This apparent contradiction can by explained by the fact that PDLLCL showed already a large amount of incorporation at 2 weeks. The increase of graft incorporation per unit of time thereafter is less compared to the other groups, although the amount of incorporation at each occasion was larger. If measurements would have been performed at the moment of operation (0 weeks), when probably no graft incorporation would have been measured in any graft, the time-effect would be more valid.

The method of fixing the grafts in the present study could have been of influence on the study. Although favourable results for membrane treatment had been demonstrated previously when the graft was not fixed,[30,31] fully rigid fixation with a micro screw would have been preferable.[32] However, titanium micro screws would have interfered with the evaluation by TMR and degradable micro screws were too large to use in this study.

In this study the new degradable barrier membrane (PDLLCL)[11] was compared to the standard non-synthetic degradable (collagen) and the standard synthetic non-degradable (ePTFE) reference materials. Although the graft of the ePTFE 12 weeks group demonstrated more resorption than the grafts in the other groups, generally all membranes tested equally compared to each other and to the control. Since the control group without a membrane performed equally well, the indication to use barrier membranes to prevent bone modeling with resorption and enhance incorporation of autologous onlay bone grafts is disputable according to our measurements.

Mineralization cannot be measured as accurately in microradiography compared to TMR, because of varying thickness of the mandible (and graft). Clear high quality pictures were obtained with TMR with higher resolutions than achievable with the current software and scanners in micro-CT. Differences in mineralization could be observed. Although only two sections per sample were examined with TMR, conclusions about graft resorption and incorporation did not differ with 3D analyses of the same samples.[27] However, TMR is time consuming compared to micro-CT. Furthermore, the section thickness of 0.50 mm, that was necessary for sufficient strength of each sample, made it impossible to visualize individual bone trabeculae and their orientation on the radiographs.

In conclusion, membranes and controls have an equal effect on bone graft modeling and incorporation in rats. It seems, therefore, that barrier membranes may not be necessary in bone grafting procedures with onlay bone block grafts in human. When particulated bone is applied, a situation that is frequently seen in clinical practice,[1] the barrier membrane is necessary to secure these granules but probably does not prevent bone resorption. For clinicians we recommended an evidence-based approach when developing a treatment plan for bone augmentation cases.[1]

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